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(54) Process for producing 5'-guanylic acid.

(57) 5'-guanylic acid (GMP) is produced by a process which comprises culturing in a medium a microorganism belonging to the genus Brevibacterium or Corynebacterium and having an ability to produce 5'-xanthylic acid (XMP) and also an ability to reproduce 5'-adenosine triphosphate (ATP) from 5'-adenylic acid (AMP); bringing a microorganism belonging to the genus Brevibacterium, Corynebacterium, Klebsiella, Bacillus, Serratia or Escherichia and having an ability to form GMP from XMP, ammonia and/or glutamine, and ATP into contact with the resulting culture broth so as to convert the XMP to GMP by coupling reaction catalyzed by the two microorganisms; and recovering GMP from the culture broth.

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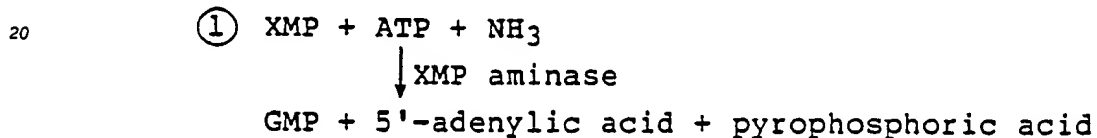
# PROCESS FOR PRODUCING 5'-GUANYLIC ACID

The present invention relates to a process for producing 5'-guanylic acid (hereinafter referred to as "GMP").

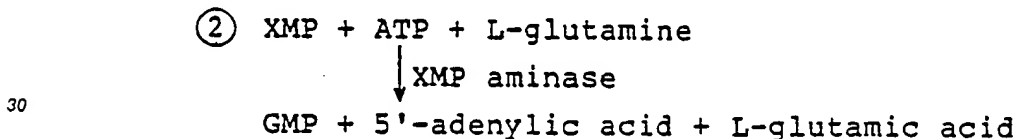
GMP is in great demand as a seasoning, and the development of a process for producing GMP at lower cost has been desired in the field of food industry.

So far known processes for producing GMP include (1) a process by enzymatically decomposing ribonucleic acid extracted from yeast cells, (2) a process by chemically phosphorylating guanosine produced by fermentation, and (3) a process by converting 5'-xanthylic acid (hereinafter referred to as "XMP") produced by fermentation to GMP by means of bacteria belonging to the genus Brevibacterium or Corynebacterium (Japanese Published Examined Patent Application No. 39069/71 corresponding to US-A-3,592,733, and Japanese Published Unexamined Patent Application No. 78697/84). Also known, EP-A-0 185 092, is a process involving the use of a transformant obtained by transforming an Escherichia coli with a recombinant DNA comprising a DNA fragment containing a gene coding for XMP aminase (GMP synthetase) involved in the formation of GMP from XMP, ammonia and/or glutamine, and 5'-adenosine triphosphate (hereinafter referred to as "ATP"), and a vector DNA (see also Japanese Published Unexamined Patent Application No. 224498/85 corresponding to EP-A-0 185 092).

The process for producing GMP from XMP by means of an enzymatic reaction is based on the following reactions catalyzed by XMP aminase:



or



Further, it is reported that the activity to convert XMP to GMP is increased by endowing a microorganism belonging to the genus Brevibacterium with a resistance to docoyinine [Biotechnol. Bioengineer., 13, 229 - 240 (1971)].

ATP is expensive, and thus a process which dispenses with addition of ATP is desirable for establishing an economical process for producing GMP by means of an enzymatic reaction. By coupling a GMP synthetic reaction system to a reaction system to reproduce ATP from 5'-adenylic acid (hereinafter referred to as AMP), and thereby using ATP repeatedly, it becomes unnecessary to add ATP to the process.

That is, the reaction to enzymatically produce GMP from XMP is carried out by coupling a reaction system for reproducing ATP from AMP by utilizing a substrate for reproducing ATP (hereinafter sometimes referred to as "ATP-reproducing system") to a reaction system for producing GMP from XMP, ammonia and/or glutamine, and ATP (hereinafter sometimes referred to as "converting reaction system").

As the ATP-reproducing system for use in the coupling reaction, a reaction system capable of reproducing ATP by utilizing a practical substrate is suitable, and, above all, the system provides an economical process for GMP production, if saccharides such as glucose, or other carbohydrates are utilized in the system. From this viewpoint, the above-mentioned processes (3) and (4) utilizing microorganisms belonging to the genus Brevibacterium or Corynebacterium, or Escherichia coli were developed. In these processes, the activities required for the two reaction systems, i.e. the activity to convert XMP to GMP and the activity to reproduce ATP, rely on a single microorganism. The process utilizing a single microorganism simultaneously having the two activities as an enzyme source for the two activities has a practical advantage of rendering the reaction system simpler, as compared with a process utilizing two kinds of microorganisms having a single activity. On the other hand, the two activities that constitute the reaction system cannot be controlled separately and independently, and thus such a problem is encountered that it is difficult to maximize the productivity by adjusting the balance between the two activities to an optimum.

In concurrence with the aforementioned problem, there is room for improvement in the processes as described in the prior art references.

That is, it is known that XMP aminase-increased strains are obtained by deriving mutants having a resistance to such chemicals as decoyinine, etc. from bacteria belonging to the genus Brevibacterium or Corynebacterium, or Escherichia coli. In the conventional process, if the amount of microorganisms is decreased according to the increase in XMP aminase activity, the ATP-reproducing activity becomes a rate-determining step. Therefore, in order to make good the shortage of ATP-reproducing activity, thereby enhancing the GMP productivity, it is necessary to use, in a relatively large amount, microorganisms serving to reproduce ATP. In case of using Escherichia coli, strains with considerably increased XMP aminase activity is obtained by genetic recombination and can be utilized. Thus, a remarkable improvement in GMP productivity is attained (Japanese Published Unexamined Patent Application No. 224498/85). However, since the ATP-reproducing activity also becomes a rate-determining step in this case also, there is still such a problem as the requirement for a relatively large amount of microorganism.

Industrial XMP-producing microorganisms are of guanine-requiring phenotype and are deficient in XMP aminase which is the XMP metabolic enzyme on the purine nucleotide biosynthetic pathway, but their ATP-reproducing activity is generally strong. In order to utilize the strong ATP-reproducing activity of the XMP-producing microorganisms as the ATP-reproducing system in the converting reaction system from XMP to GMP, the present inventor has investigated processes for producing GMP from XMP by combining the ATP-reproducing activity with the XMP aminase activity of various microorganisms in the development of a new process for producing GMP. If and when such a process is established, waste cells of the XMP-producing microorganism, which have so far been neglected as useless ones, can be utilized as an enzyme source with the ATP-reproducing activity, and also the above-mentioned problem of failing to separate the two activities of ATP-reproducing system and converting reaction system from each other can be solved, whereby the optimization of the reaction system can be made easily. Concurrently, when the XMP aminase activity of the converting microorganism is increased by certain means, the amount of the converting microorganism to be used in the conversion reaction can be decreased in accordance with the degree of the increase in the XMP aminase activity, irrespective of the degree of the ATP-reproducing activity, and thus the amount of raw materials for culturing the converting microorganism can considerably be decreased per GMP produced. That is, it is expected to set forth the merit of increase in conversion activity.

In accordance with the present invention, an economical process for producing GMP is provided. This process comprises culturing in a medium a first microorganism belonging to the genus Brevibacterium or Corynebacterium and having an ability to produce XMP and also an ability to reproduce ATP from AMP, and referred to hereinafter as the XMP-producing microorganism; bringing into contact with the culture broth during the culturing or after completion of the culturing of the first microorganism a second microorganism having an ability to form GMP from XMP, ammonia, and/or glutamine, and ATP, and hereinafter referred to as the converting microorganism, thereby to convert XMP to GMP by a coupling reaction catalyzed by the two microorganisms; and recovering GMP from the culture broth. The amount of the converting microorganism can be decreased by having both an XMP-producing microorganism and a converting microorganism concurrently coexist and utilizing the ATP-reproducing activity of the XMP-producing microorganism. Thus, the yield of GMP per raw materials can be increased by the consequent decrease in the necessary amount of the raw materials for culturing the converting microorganism.

Suitable XMP-producing microorganisms having ATP-reproducing activity suitable for the process herein include, for example, Brevibacterium ammoniagenes ATCC 21076, Brevibacterium ammoniagenes ATCC 21154, Brevibacterium ammoniagenes X-21 ATCC 21263, Corynebacterium glutamicum ATCC 15135, Corynebacterium glutamicum X-31 ATCC 21265, etc. By culturing these microorganisms according to an ordinary culturing procedure, a considerable amount of XMP can be accumulated in the medium. That is, these microorganisms are cultured in an ordinary medium containing appropriate carbon sources, appropriate nitrogen sources, appropriate inorganic materials, amino acids, vitamins, etc., under aerobic conditions, while adjusting the temperature, pH, etc.

As the carbon source, carbohydrates such as glucose, fructose, sucrose, maltose, etc.; sugar alcohols such as mannitol, sorbitol, glycerol, etc.; various organic acids such as pyruvic acid, lactic acid, citric acid, etc.; and various amino acids such as glutamic acid, methionine, lysine, etc. can be used. Furthermore, natural organic nutrient sources such as starch hydrolyzate, molasses, rice bran, cassava, bagasse, corn steep liquor, etc. can also be used.

As the nitrogen source, ammonia; various inorganic and organic ammonium salts as ammonium chloride, ammonium sulfate, ammonium carbonate, ammonium acetate, etc.; amino acids such as glutamic acid, glutamine, methionine, etc.; and nitrogen-containing organic materials such as peptone, NZ-amine, corn steep liquor, meat extract, yeast extract, casein hydrolyzate, fish meal or its digested product, etc. can be used.

Furthermore, as the inorganic materials, potassium dihydrogen phosphate, sodium monohydrogen phosphate, magnesium sulfate, sodium chloride, calcium chloride, iron chloride, copper sulfate, manganese chloride, ammonium molybdate, zinc sulfate, etc. can be added to the medium, if required. Vitamins, amino acids, nucleic acids, etc. to be required for the growth of microorganisms are added to the medium, if necessary. Naturally, they are supplied by other medium components described above, it is not necessary to add these specific nutrients separately to the medium.

Culturing is generally carried out under aerobic conditions, for example, by shaking or by aeration and agitation. The preferred culturing temperature is generally 20 to 40°C, more preferably 25 to 35°C; and the pH of the medium is preferably maintained around neutrality during the culturing. Culturing period is usually 10 to 120 hours.

As the converting microorganism to be used in the present invention, any microorganism can be used, so long as it has an XMP aminase activity of forming GMP from XMP, ammonia and/or glutamine, and ATP. The following strains are exemplary of those suitable for the converting strain.

- Brevibacterium ammoniagenes ATCC 6872
- Corynebacterium glutamicum ATCC 13059
- Corynebacterium glutamicum ATCC 31834
- Klebsiella pneumoniae ATCC 8308
- Klebsiella pneumoniae ATCC 9621
- Bacillus pumilus ATCC 14884
- Bacillus subtilis ATCC 14617
- Serratia marcescens ATCC 13880
- Serratia marcescens ATCC 264
- Escherichia coli B ATCC 11303
- Escherichia coli B ATCC 23226
- Escherichia coli K-12 ATCC 10798
- Escherichia coli K-12 ATCC 14948
- Escherichia coli K294 (r<sup>-</sup>, m<sup>+</sup>) FERM BP-526

Strains whose XMP aminase activity is increased by a conventional mutagenesis such as drug-resistant mutation, etc. can also be used. Furthermore, strains whose XMP aminase activity is increased by molecular breeding procedure such as genetic recombination, cell fusion, etc. of bacteria belonging to the genus Brevibacterium, Corynebacterium, Escherichia or Bacillus can also be used. For example, Brevibacterium ammoniagenes ATCC 21170, which is a strain having high XMP aminase activity, selected from decoyinine-resistant mutants derived from Brevibacterium ammoniagenes ATCC 6872 by the nitrosoguanidine treatment, Brevibacterium ammoniagenes ATCC 21264, which is a mutant having high XMP aminase activity derived by the ultraviolet treatment, etc. or Escherichia coli K294/pXA10, FERM BP-499 whose XMP aminase activity is increased by transformation with a plasmid wherein a gene coding for the XMP aminase (guaA) of Escherichia coli K-12 strain is inserted into pBR322 as a vector of Escherichia coli, etc. can be used. The detailed procedures for construction of plasmid pXA10 obtained by inserting a DNA fragment containing guaA of Escherichia coli chromosome into plasmid pBR322 at a tetracycline-resistant site, and inserting a tryptophan promoter to an upstream site of guaA, and construction of Escherichia coli K294/pXA10 FERM BP-499 obtained by transformation of the strain K294 with pXA10, are disclosed in Japanese Published Unexamined Patent Application No. 224498/85 (EP-A-0 185 092).

A culture liquor, cells, or then treated products having a strong activity of forming GMP from XMP, ammonia and/or glutamine, and ATP can be obtained by culturing these microorganisms according to an ordinary culturing procedure. That is, these microorganisms are cultured in an ordinary medium containing an appropriate carbon source, an appropriate nitrogen source, appropriate inorganic materials, amino acids, vitamins, etc. under aerobic conditions while adjusting the temperature, pH, etc.

As the carbon source, carbohydrates such as glucose, fructose, sucrose, maltose, etc.; sugar alcohols such as mannitol, sorbitol, glycerol, etc.; various organic acids such as pyruvic acid, lactic acid, citric acid, etc.; and various amino acids such as glutamic acid, methionine, lysine, etc. can be used. Furthermore, natural organic nutrient sources such as starch hydrolyzate, molasses, rice bran, cassava, bagasse, corn steep liquor, etc. can also be used.

As the nitrogen source, ammonia; various inorganic and organic ammonium salts such as ammonium chloride, ammonium sulfate, ammonium carbonate, ammonium acetate, etc.; amino acids such as glutamic acid, glutamine, methionine, etc.; and nitrogen-containing organic materials such as peptone, NZ-amine, corn steep liquor, meat extract, yeast extract, casein hydrolyzate, fish meal or its digested product, etc. can be used.

Furthermore, as the inorganic materials, potassium dihydrogen phosphate, sodium monohydrogen phosphate, magnesium sulfate, sodium chloride, calcium chloride, iron chloride, copper sulfate, manganese chloride, ammonium molybdate, zinc sulfate, etc. can be added to the medium, if required. Vitamins, amino acids, nucleic acids, etc. to be required for the growth of microorganisms are added to the medium, if necessary. Naturally, if they are supplied by other medium components described above, it is not necessary to add these specific nutrients separately to the medium. In case of using *E. coli* K294/pXA10, FERM BP-499, a medium in which the tryptophan content is low (less than 5 mg/l) can achieve a good result.

Culturing is generally carried out under aerobic conditions, for example, by shaking or by aeration and agitation. The preferred culturing temperature is generally 20 to 50°C, more preferably 28 to 45°C, and the pH of the medium is preferably maintained around neutrality during the culturing. Culturing period is usually 1 to 48 hours.

The thus obtained fermentation liquor containing XMP and the culture liquor of the converting microorganism having an ability to convert XMP to GMP can be mixed after completion of the individual culturing of the two kinds of the microorganisms, or the culture liquor of the converting microorganism may be added to the medium for XMP fermentation at any time from the start to the completion of the XMP fermentation or the XMP fermentation liquor may be added to the medium for culturing the converting microorganism at any time from the start to the completion of culturing the converting microorganism. Furthermore, the XMP-producing microorganism and the converting microorganism can be cultured in a single medium at the same time, and the resulting culture liquor can be used.

To the thus obtained culture broth containing XMP, XMP-fermenting cells and converting cells are added ammonia and/or glutamine, and an ATP-reproducing substrate immediately or after various treatments of the culture broth. The treated culture broth includes, for example, concentrated or dried culture broth, cells obtained by centrifuging the culture broth, dried cells, acetone-treated product, surfactant and/or organic solvent-treated product, lytic enzyme-treated product, immobilized cells or extracted enzyme preparation from cells, etc.

Conversion of XMP to GMP is carried out by adding phosphate ion, magnesium ion, and a surfactant and/or an organic solvent, if required, to the said mixture, while adjusting the pH to 6 - 10, preferably 7 - 8 and keeping the temperature at 20 to 50°C for 1 to 48 hours. The concentration of XMP during the conversion of XMP to GMP is desirably in a range of 1 to 100 mg/ml in terms of  $\text{XMP} \cdot \text{Na}_2 \cdot 7\text{H}_2\text{O}$ , which is hereinafter applied to express the concentration.

Any of carbohydrates such as glucose, arabinose, lactose, maltose, sucrose, mannitol, sorbitol, trehalose, starch hydrolyzate, etc.; organic acids such as pyruvic acid, lactic acid, acetic acid,  $\alpha$ -ketoglutaric acid, etc.; amino acids such as glycine, alanine, aspartic acid, glutamic acid, etc. can be used as the ATP-reproducing substrate, so long as it can be utilized by the XMP-producing microorganism. Furthermore, phosphorylated compounds such as sodium or potassium salt of acetylphosphoric acid, carbamylphosphoric acid, creatinephosphoric acid, etc. can also be used.

Any of cationic surfactants such as polyoxyethylenestearylamine (for example, Nimiin S-215, product of Nihon Yushi), cetyltrimethylammonium bromide, etc.; anionic surfactants such as sodium oleylamide sulfate, etc.; and amphoteric surfactants such as polyoxyethylenesorbitan monostearate (for example, Nonion ST221, product of Nihon Yushi), etc. can be used as the surfactant, so long as it can promote conversion of XMP to GMP, and can usually be used in a concentration of 0.1 to 50 mg/ml, preferably 1 to 20 mg/ml.

As the organic solvent, toluene, xylene, aliphatic alcohols, benzene, ethyl acetate, etc. can be used in a concentration of 0.1 to 50  $\mu\text{l/ml}$ , preferably 1 to 20  $\mu\text{l/ml}$ .

During the conversion of XMP to GMP, the concentration of phosphate ion and magnesium ion is desirably kept in a range of 4 to 400 mM. When the amount of these ions to be brought into the conversion system by the medium or cells falls within this concentration range, it is not necessary to add these ions to the conversion system, whereas in case these ions are short, they are added thereto so that the amount may fall within the said concentration range. As the phosphate ions, any of sodium salt, potassium salt, magnesium salt, etc. of phosphoric acid can be used. As the magnesium salt, any of inorganic and organic salts can be used.

As the source for ammonia and/or glutamine, any of ammonia gas, various inorganic and organic ammonium salts, glutamine, and glutamine-containing natural products such as yeast extract, casamino acid, corn steep liquor, etc. can be used.

Recovery of GMP from the culture broth can be carried out according to an ordinary procedure using activated carbon, ion exchange resin, etc.

Certain specific embodiments of the present invention are illustrated by the following representative examples.

#### 10 Example 1

One loopful of Escherichia coli K294 (FERM BP-526) strain was inoculated in a sterilized 70 ml-large test tube containing 10 ml of a seed medium (pH 7.2) comprising 1% polypeptone, 0.5% meat extract, 0.5% yeast extract and 0.25% sodium chloride, and cultured at 30°C for 16 hours by reciprocative shaker (280 reciprocation/min.). Then, 2 ml of the cultured seed medium was inoculated in a 1 l-Erlenmeyer flask containing 200 ml of M9 medium (pH 7.4) consisting of 6 mg/ml  $\text{Na}_2\text{HPO}_4$ , 3 mg/ml  $\text{KH}_2\text{PO}_4$ , 5 mg/ml  $\text{NaCl}$ , 1 mg/ml  $\text{NH}_4\text{Cl}$ , 4  $\mu\text{g/ml}$  thiamine $\cdot\text{HCl}$ , 3 mg/ml glucose and 0.25 mg/ml  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , 2 mg/ml casamino acid, and cultured at 30°C for 16 hours by rotary shaker (220 rpm). The cells were collected by centrifugation at 13,000 x g for 20 minutes and frozen at -20°C for reservation.

One loopful of Brevibacterium ammoniagenes ATCC 21154 strain was inoculated in a sterilized large test tube containing 10 ml of a seed medium consisting of 1% polypeptone, 0.5% meat extract, 0.5% yeast extract and 0.25% sodium chloride (pH 7.2) and cultured at 30°C for 24 hours by reciprocative shaking (280 reciprocation/min.). Then, 2ml of the cultured seed medium was inoculated in 300 ml-Erlenmeyer flasks provided with baffles each containing 20 ml of a medium consisting of 15% glucose, 0.01% casein hydrolyzate, 0.7% yeast extract, 1.0% ammonium sulfate, 0.3%  $\text{KH}_2\text{PO}_4$ , 0.3%  $\text{K}_2\text{HPO}_4$ , 0.5%  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , 10  $\mu\text{g/ml}$  each of adenine and guanine, and 10  $\mu\text{g/l}$  of biotin (pH 7.2), autoclaved at 120°C for 20 minutes, and cultured at 30°C by rotary shaker (220 rpm), while the pH was kept approximately at pH 7 by adding urea to the medium, if necessary. At 92nd hour from the start of culturing 38.0 mg/ml XMP was formed.

The frozen Escherichia coli cells were suspended in water and added to the XMP fermentation liquore so that the wet cell concentration may be 7.5 mg/ml. Then, 50 mg/ml glucose, 2 mg/ml sodium phytate, 5 mg/ml  $\text{Na}_2\text{HPO}_4$  and 5 mg/ml  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , and further 4 mg/ml Nimiin S-215 and 10  $\mu\text{l/ml}$  xylene were added thereto. Then, 20 ml of the thus obtained mixture was poured to each of 200 ml-beakers, and stirred by a magnetic stirrer at 900 rpm to conduct the conversion reaction of XMP to GMP at 42°C for 24 hours while adjusting the pH to about 7.4 by adding aqueous ammonia thereto. As a result, 6.5 gm/ml GMP in terms of  $\text{GMP}\cdot\text{Na}_2\cdot 7\text{H}_2\text{O}$ , (which is hereinafter applied to express the concentration) was formed and accumulated. In case of adding neither Nimiin S-215 nor xylene, 1.8 mg/ml GMP was formed and accumulated. In case of adding no Escherichia coli cells and in case of a centrifuged supernatant in place of the XMP fermentation liquor, GMP was not substantially formed.

#### Example 2

The same procedure was repeated under the same conditions as in Example 1, except that Escherichia coli K294/pXA10 (FERM BP-499) was used in place of Escherichia coli K294 (FERM BP-526). It was found that 32.0 mg/ml GMP was formed and accumulated after 23 hours.

#### Example 3

The same procedure was repeated under the same conditions as in Example 1. except that Corynebacterium glutamicum X-31 ATCC 21265 was used as the XMP-producing microorganism. It was found 12 mg/ml XMP and 6.1 mg/ml GMP were formed.

Example 4

Culturings of Escherichia coli and Brevibacterium ammoniagenes were carried out in the same manner as in Example 1, except that Escherichia coli K294/pXA10 was used in place of Escherichia coli K294. In place of frozen Escherichia coli cells, 2 ml of Escherichia coli culture broth was added to 20 ml of Brevibacterium ammoniagenes culture broth containing 33 mg/ml XMP, and conversion of XMP to GMP was carried out in the same manner as in Example 1. As a result, 23.1 mg/ml GMP was formed.

10 Example 5

Reaction (A) was carried out in the same manner as in Example 1 except that the respective strains shown in Table 1 were used as the converting strain, and reaction (B) was carried out in the same manner as in Example 1, except that the respective strains shown in Table 1 were used as the converting strain and that the centrifuged supernatant of XMP fermentation liquor was used in place of the XMP fermentation liquor itself. Results are shown in Table 1. The yield of XMP was 36.9 mg/ml.

Table 1  
Production of GMP by combinations of XMP aminase activity  
of various microorganisms with ATP-reproducing activity of  
XMP-producing microorganisms

| Strains                               |             | GMP<br>(A) | (mg/ml)<br>(B) |
|---------------------------------------|-------------|------------|----------------|
| <u>Brevibacterium ammoniagenes</u>    | ATCC 6872   | 6.7        | 0.8            |
| <u>B. ammoniagenes</u>                | ATCC 21170  | 12.3       | 0.6            |
| <u>B. ammoniagenes</u>                | ATCC 21264  | 13.5       | 0.7            |
| <u>Corynebacterium glutamicum</u>     | ATCC 13059  | 6.1        | 0.3            |
| <u>C. glutamicum</u>                  | ATCC 31834  | 4.6        | 0.3            |
| <u>Klebsiella pneumoniae</u>          | ATCC 8308   | 3.3        | 0.2            |
| <u>K. pneumoniae</u>                  | ATCC 9621   | 5.5        | 0.5            |
| <u>Bacillus pumilus</u>               | ATCC 14884  | 6.6        | 0.4            |
| <u>Bacillus subtilis</u>              | ATCC 14617  | 5.0        | 0.5            |
| <u>Serratia marcescens</u>            | ATCC 13880  | 7.3        | 0.9            |
| <u>S. marcescens</u>                  | ATCC 264    | 4.9        | 0.7            |
| <u>Escherichia coli</u> B             | ATCC 11303  | 6.3        | 0.5            |
| <u>E. coli</u> B                      | ATCC 23226  | 3.8        | 0.2            |
| <u>E. coli</u> K-12                   | ATCC 10798  | 3.4        | 0.4            |
| <u>E. coli</u> K-12                   | ATCC 14948  | 5.5        | 0.3            |
| <u>E. coli</u> K294 ( $r^-$ , $m^+$ ) | FERM BP-526 | 7.2        | 0.4            |



Relevant deposit details of microorganisms referred to hereinbefore are as follows:

Escherichia coli K294/pXA10 deposited 8th March 1984 under the terms of the Budapest Treaty with the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, Deposit No. FERM BP-499.

- 5 Escherichia coli K294 similarly deposited with the same agency and on the same terms, deposit date 20th April 1984, deposit No. FERM BP-526.

Corynebacterium glutamicum, L 15 deposited 9th March 1981 under the terms of the Budapest Treaty with the American Type Culture Collection, Rockville, Maryland, USA, Deposit No. ATCC 31834.

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## Claims

1. A process for the production of 5'-guanylic acid (GMP) which comprises culturing a microorganism capable of converting 5'-xanthylic acid (XMP) into GMP in a culture medium containing XMP, thereby to  
15 accumulate GMP in the culture medium, and recovering the product GMP from the medium, characterised by culturing in a culture medium a first microorganism belonging to the genus Brevibacterium or Corynebacterium and having the ability to produce XMP in said medium as well as the ability to reproduce 5'-adenosine triphosphate (ATP) from 5'-adenylic acid (AMP), and during or after such culturing, bringing into contact with the XMP-containing culture medium a second microorganism, said second microorganism  
20 having the ability to produce GMP from XMP, ammonia and/or glutamine and ATP, thereby converting the XMP in said medium into GMP by a coupling reaction catalysed by the two microorganisms.

2. A process according to claim 1, characterised in that the second microorganism is a bacterium belonging to the genus Brevibacterium, Corynebacterium, Klebsiella, Bacillus, Serratia or Escherichia.

3. A process according to claim 1, characterised in that the second microorganism is a transformant  
25 obtained by transforming a microorganism with a recombinant DNA comprising a DNA fragment containing a gene coding for 5'-xanthylic acid aminase (5'-guanylic acid synthetase) and a vector DNA.

4. A process according to claim 3, characterised in that the microorganism to be transformed belongs to Escherichia coli.

5. A process according to claim 4, characterised in that the second microorganism is Escherichia coli  
30 K294/pXA10, FERM BP-499.

6. A process according to claim 1, characterised in that the second microorganism is a decoyinine-resistant mutant belonging to the genus Brevibacterium or Corynebacterium.

7. A process according to claim 2, characterised in that the second microorganism is  
Brevibacterium ammoniagenes ATCC 6872  
35 Corynebacterium glutamicum ATCC 13059  
Corynebacterium glutamicum ATCC 31834  
Klebsiella pneumoniae ATCC 8308  
Klebsiella pneumoniae ATCC 9621  
Bacillus pumilus ATCC 14884  
40 Bacillus subtilis ATCC 14617  
Serratia marcescens ATCC 13880  
Serratia marcescens ATCC 264  
Escherichia coli B ATCC 11303  
Escherichia coli B ATCC 23226  
45 Escherichia coli K-12 ATCC 10798  
Escherichia coli K-12 ATCC 14948  
or Brevibacterium ammoniagenes ATCC 21264.

8. A process according to any one of claims 1-7, characterised in that the first microorganism is  
Brevibacterium ammoniagenes ATCC 21076  
50 Brevibacterium ammoniagenes ATCC 21154  
Brevibacterium ammoniagenes X-21 ATCC 21263  
Corynebacterium glutamicum ATCC 15135  
or Corynebacterium glutamicum X-31 ATCC 21265.

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(54) Process for producing 5'-guanylic acid.

(57) 5'-guanylic acid (GMP) is produced by a process which comprises culturing in a medium a microorganism belonging to the genus Brevibacterium or Corynebacterium and having an ability to produce 5'-xanthylic acid (XMP) and also an ability to reproduce 5'-adenosine triphosphate (ATP) from 5'-adenylic acid (AMP); bringing a microorganism belonging to the genus Brevibacterium, Corynebacterium, Klebsiella, Bacillus, Serratia or Escherichia and having an ability to form GMP from XMP, ammonia and/or glutamine, and ATP into contact with the resulting culture broth so as to convert the XMP to GMP by coupling reaction catalyzed by the two microorganisms; and recovering GMP from the culture broth.

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| DOCUMENTS CONSIDERED TO BE RELEVANT   |   |   |   |
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| Category  | Citation of document with indication, where appropriate, of relevant passages   | Relevant to claim   | CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)                |
| X   | CHEMICAL ABSTRACTS, vol. 76, no. 7, 14th February 1972, page 218, no. 32874y, Columbus, Ohio, US; & JP-A-71 39 070 (KYOWA FERMENTATION INDUSTRY CO., LTD) 17-11-1971<br>* Abstract *  | 1,2   | C 12 P 19/32<br>C 12 P 39/00 //<br>C 12 N 15/00               |
| Y   | IDEM<br>---   | 3-8   |   |
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| The present search report has been drawn up for all claims  |   |   |   |
| Place of search<br>THE HAGUE  |   | Date of completion of the search<br>19-10-1988  | Examiner<br>PULAZZINI A.F.R.                                  |
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